

Application No.: 10/574,380
Attorney Docket No.: 81197-002US0
First Applicants' Name: Andreas M. Zeiher
Application Filing Date: 09 March 2007
Office Communication Dated: 10 August 2009
Date of Response: 10 December 2009
Examiner: Michail A. Belyavskyi

IN THE CLAIMS:

Applicants, pursuant to 37 CFR § 1.121, submit the following amendment to the Claims:

1. (Previously presented) An in vitro method for analyzing a sample from a mammal in connection with at least one cardiovascular disease, wherein said method comprises the following steps:
 - a) isolating bone marrow-precursor-cells (BMP) and/or blood-derived circulating precursor-cells (BDP) by means of at least one cell specific surface marker, and
 - b) determining the cardiovascular functionality of the isolated BMP and/or BDP by means of a migration assay.
2. (Previously presented) The method according to claim 1, further comprising the comparison of the result as obtained from the sample as examined with a reference value and/or the result of a reference sample.
3. (Previously presented) The method according to claim 1, wherein the sample to be examined is derived from a human.
4. (Previously presented) The method according to claim 1, wherein the sample to be examined is selected from the group consisting of bone marrow, peripheral blood or fractions thereof and cell culture-susensions or fractions thereof.
5. (Previously presented) The method according to claim 4, wherein a coagulation inhibitor is added to the peripheral blood.
6. (Previously presented) The method according to claim 4, wherein the sample to be examined is obtained by means of punctuation from the bone marrow.
7. (Previously presented) The method according to claim 1, wherein the isolating occurs by using density-gradient-centrifugation, cell specific surface markers, and/or immunological methods.

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8. (Previously presented) The method according to claim 7, wherein the isolating occurs by using FACS or immunomagnetic separation.

9. (Previously presented) The method according to claim 1, wherein the cell specific surface marker for BMP is selected from CD34, CD45 and CD133, and for BDP is selected from VEGFR2, CD105, vWF and CD31.

10. (Previously presented) The method according to claim 1, wherein the migration assay is performed in a Boyden-chamber or a modified Boyden-chamber.

11. (Previously presented) The method according to claim 1, wherein the migration assay is performed using SDF-1, VEGF, P1GF or MCP-1.

12. (Previously presented) The method according to claim 1, wherein the cardiovascular disease is selected from the group consisting of stable and unstable angina, stable coronary heart disease, acute coronary syndrome, myocardial infarction, acute myocardial infarction, acute heart syndrome, coronary artery disease, chronic ischemic cardiomyopathy (ICMP), dilatative cardiomyopathy (DCM), heart insufficiency, and other causes of a cardiac weakness.

13. (Previously presented) The method according to claim 1, wherein the method is performed immediately before a cell infusion into the mammal.

14. (Previously presented) The method according to claim 1, wherein the examined isolated BMP and/or BDP are autologous and/or heterologous for the mammal.

15. (Previously presented) A diagnostic kit, comprising means for performing an in vitro method for analyzing a sample from a mammal in connection with at least one cardiovascular disease, wherein said method comprises the following steps:

a) isolating bone marrow-precursor-cells (BMP) and/or blood-derived circulating precursor-cells (BDP) by means of at least one cell specific surface marker, and

b) determining the cardiovascular functionality of the isolated BMP and/or BDP by means of a migration assay, optionally together with additional components and/or excipients.

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16. (Previously presented) A method for diagnosing and/or prognosing cardiovascular diseases, for monitoring of therapies and/or for the determination of the cardiovascular functionality of BMPs or BDPs for a stratification for a prospective cell therapy with stem- and progenitor cells for increasing the perfusion of ischemic tissue or for the regeneration of tissue loss in particular in heart insufficiency, and/or for identifying patients that would profit from an ex vivo pretreatment of their BMPs or BDPs for an improvement of the cardiovascular functionality before retransplantation of the cells.

17. (Currently amended) An in vitro method for isolating bone marrow-precursor-cells (BMPs) and/or blood-derived circulating precursor-cells (BDPs), comprising:

- a) taking a sample from a donor-mammal;[[.]]
- b) isolating BMPs and/or BDPs from the sample so obtained;[[.]] and
- c) determining the cardiovascular functionality of the isolated BMPs and/or BDPs by means of a migration assay.

18. (Previously presented) The method according to claim 17, wherein the sample to be examined is derived from a human.

19. (Previously presented) The method according to claim 17, wherein the sample to be examined is selected from the group consisting of bone marrow, peripheral blood or fractions thereof and cell culture-suspensions or fractions thereof.

20. (Previously presented) The method according to claim 19, wherein the sample to be examined is obtained by means of punctuation from the bone marrow.

21. (Previously presented) The method according to claim 17, wherein the isolating occurs by means of density-gradient-centrifugation, cell specific surface markers, and/or immunological methods.

22. (Previously presented) The method according to claim 21, wherein the isolating occurs by using FACS or immunomagnetic separation.

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23. (Previously presented) The method according to claim 17, wherein said isolating step utilizes a cell specific surface marker for BMP selected from CD34, CD45 and CD133, and for BDP selected from VEGFR2, CD105, vWF and CD31.

24. (Previously presented) The method according to claim 17, wherein the migration assay is performed in a Boyden-chamber or a modified Boyden-chamber.

25. (Previously presented) The method according to claim 17, wherein the migration assay is performed by using SDF-1, VEGF, P1GF or MCP-1.

26. (Previously presented) The method according to claim 17, wherein the isolated BMP and/or BDP are genetically modified, in order to improve the cardiovascular functionality of the cells.

27. (Currently amended) A bone marrow-precursor-cell (BMP) or blood-derived circulating precursor-cell (BDP), obtained according to an in vitro method for isolating specific bone marrow-precursor-cells (BMPs) and/or blood-derived circulating precursor-cells (BDPs), comprising:

- a) taking a sample from a donor-mammal;[.,.]
- b) isolating BMPs and/or BDPs from the sample so obtained;[.,.] and
- c) determining the cardiovascular functionality of the isolated BMPs and/or BDPs by means of a migration assay.

28. (Previously presented) The bone marrow-precursor-cell (BMP) or blood-derived circulating precursor-cell (BDP) according to claim 27, wherein the isolated BMPs and/or isolated BDPs are autologous and/or heterologous for the mammal.

29. (Currently amended) A method for producing a pharmaceutical composition, comprising obtaining a sample from a mammal, wherein said obtaining comprises the following steps:

- a) isolating bone marrow-precursor-cells (BMP) and/or blood-derived circulating precursor-cells (BDP) by means of at least one cell specific surface marker;[.,.] and

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b) determining the cardiovascular functionality of the isolated BMP and/or BDP by means of a migration assay and furthermore formulating said pharmaceutical composition by admixing with pharmaceutically acceptable carries and/or diluents.

30. (Previously presented) The method according to claim 29, wherein formulating furthermore comprises an admixing with a statin, VEGF and/or erythropoietin.

31. (Currently amended) A pharmaceutical composition, produced by obtaining a sample from a mammal, wherein said obtaining comprises the following steps:

a) isolating bone marrow-precursor-cells (BMP) and/or blood-derived circulating precursor-cells (BDP) by means of at least one cell specific surface marker;[[,]] and

b) determining the cardiovascular functionality of the isolated BMP and/or BDP by means of a migration assay and furthermore formulating said pharmaceutical composition by admixing with pharmaceutically acceptable carries and/or diluents.

32. (Currently amended) A method for the treatment of a cardiovascular disease, selected from the group consisting of stable and unstable angina, stable coronary heart disease, acute coronary syndrome, myocardial infarction, acute myocardial infarction, acute heart syndrome, coronary artery disease, chronic ischemic cardiomyopathy (ICMP), dilatative cardiomyopathy (DCM), heart insufficiency, and other causes of a cardiac weakness, wherein said method comprises administering to a mammal in need of such treatment, a bone marrow-precursor-cell (BMP) or blood-derived circulating precursor-cell (BDP), obtained by an in vitro method comprising:

a) taking a sample from a donor-mammal;[[,]]

b) isolating BMPs and/or BDPs from the sample so obtained;[[,]] and

c) determining the cardiovascular functionality of the isolated BMPs and/or BDPs by means of a migration assay.

33. (Previously presented) The method according to claim 32, wherein the treatment comprises the infusion of cells into the mammal.

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34. (Previously presented) The method according to claim 32, wherein the treatment furthermore comprises the administration of statines, in particular atorvastatin, VEGF, and/or erythropoietin.